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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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PROVOCATION OF AN INFECTION VIA PROTECTIVE VACCINATION

I. Prevention of a provocation of a virus infection by oral vaccination with inactivated virus during the incubation period.

(Following is the translation of an article by H. Raettig, Dept. of Bacteriology and Infectious Diseases. Robert Koch Institute, Berlin, which appeared in the German language periodical Zbl. Bact., Orig. 200: pages 448-459, (1966). Translation performed by Constance L. Lust.)

Vaccination with inactivated microorganisms has received renewed importance recently in basic research and in immunology practice. Their wide spectrum of effectiveness, rapid effect and easy preparation of this method is to be recommended particularly in catastrophes (1). In these cases (war, earthquakes, floods), even when vaccination is carried out fast, many people could already have been in contact with the germ. It, thus, was of importance to test if with an oral vaccine the danger of provocation exists during the incubation period, as was demonstrated to be the case for the parenteral method of vaccination (2). Therefore this aspect was tested in a model infection of the mouse with S. typhimurium (3). The results of these earlier experiments showed that oral vaccination does not have undesirable effects during the incubation period, but, rather appears to be protective. In the poliovirus-mouse model after inter-cerebral injection a provocative effect was illicited by parenteral vaccination during the incubation period (4). In 1962 it could not be tested whether this could be avoided by oral immunization, because at that time no oral vaccine for a virus was available. In the meantime success was obtained in protecting mice against oral, virulent infections of Col. SK virus (5) by oral vaccination with an inactivated virus. It now had to be tested if this vaccination method could be used without damage during the incubation time for our infection model system. We tested this in two virus-models with white mice - oral infection with Col. SK virus and cerebral infection with the MEF strain of poliomyelitis virus.

Methods and Materials

Animals: We used 1500 white mice of the NMRI strain. All mice weighed 8-10 grams and were 3-4 weeks old.

Infection: Col. SK oral, with 0.2 ml 10^{-3} dilution of infected mouse brain suspensions. Our experience (6) is that in this model 50-80% die. In the other model the mice were injected I.C. with a mouse-adapted strain of type II (Lansing) MEF strain of polio. They received 0.03 ml 10^{-2} dilution of infected mouse brain. Vaccine was made as described in reference (4).

Provocation:

Model 1. Mice were immunized specifically or unspecifically 1-2 days after infection with virulent Col. SK virus. Specific = 0.25 ml self made vaccine from mouse brains that had been infected with Col. SK. Brain was homogenized in 1:10 saline and the supernatant liquid was inactivated for 60 minutes at 60°C. To test that this preparation contained no viruses young mice were injected I.C. Model 2. On day 5 or, 5x from day 3-7 mice were vaccinated orally (after IC infection) with a commercial (Salk method) prep of polio vaccine (from Behringwerke, Marburg). (We thank Prof. Hennessen for this vaccine).

Calculation of data: The effect of the oral vaccine during the incubation period was demonstrated as in previous reports. The mortality of the mice is presented graphically, and the end result is presented in a table. Mortality is defined in this work as: We immediately killed all animals that were paralized, because we know they would die within 24 hours. The day of mortality was therefore the day of paralysis. The life span is shortened but we can neglect this because all conditions are standardized.

Results

Specific, oral incubation-vaccination after Col SK infection.

The incubation time of oral Col.SK is very short in the mouse; about 2.5 days when using virus adapted for oral infection. Mice that became ill die on day 2-3 after infection. The most inappropriate time to actively immunize parenterally was the late incubation period, according to epidemiological-statistical experience (7) and from animal trials (8). Therefore animals were given oral vaccine 2 days post inoculation in order to test the most severe conditions (trial #144 and 145).

In the first run, #144 (see table 1) on day 2 post inoculation, at the time of provocation vaccination, 34% of the mice were dead; the high final mortality, 84%, can only be partly attributed to vaccination during incubation. In #145 at the "incubation-time" only 10% had died. The vaccination could still protect animals, as may be seen in the low final mortality of 42%, as compared to controls (table 1,row 5). In trials 146, 148, 149 immunization was done on day 1 post inoculation. Values are compared for total mortality in rows 5-7 of table 1. It shows that vaccination with Col. SK-vaccine does not influence unfavorably the course of infection as measured by mortality.

The average values of 5 individual experiments (table 1) show almost identical values for controls and animals vaccinated during the incubation time. Total mortality values were 63.7 and 62.8% for the middle death times of 2.56-2.61 days. The course of mortality was not provoked by specific oral vaccination. This is illustrated in figure 1.

Unspecific, oral incubation-vaccination after Col. SK infection.

In earlier work (5) it was demonstrated that oral administration of

inactivated virus, which was not serologically related to Col. SK, protected against oral infection. We used for these unspecific vaccinations a commercial, formaldehyde-inactivated vaccine according to Salk. Since oral vaccination with heterologous vaccine protected, it was theoretically possible that it harms during the incubation period. We immunized mice with polio vaccine on day 1 after oral infection with Col. SK. The results of these experiments are presented in rows 6 and 9 of table 1. In 146, 148, 149 the unspecific provocation ran about the same as the specific. The values of table 1 show mortality and time of death of the animals vaccinated during incubation does not deviate significantly from the values of the controls. The mean values of controls and vaccinated animals for mortality are 61.7-63.4% at 2.40 days. As illustrated graphically in figure 2 the data shows now that the course of mortality has no differences. Therefore, the unspecific, oral vaccination does not provoke either during the incubation period in this model system.

Specific, oral incubation vaccination after IC infection with poliomyelitis.

Previously (4) we showed that young mice infected IC with Lansing type MEF and then inoculated (subqutaneously) during the incubation period with commercial Salk vaccine, died earlier and had a higher mortality rate than non-immunized controls. On this model we also tested whether the provocation due to the specific subqutaneously inoculation during incubation could be avoided by using vaccine orally. The data of 6 experiments are presented in table 6 and the following figures.

In experiments 141, 143, 151, and 153 the differences in mortality of vaccinated and non-vaccinated animals are very small (table 2). The middle death time in this case provides no indication as to provocation. Trial 143 is presented graphically in figure 3. This also indicates no provocation. Of special interest is experiment 152. In this case mortality was already lower than controls before the incubation period. It never increased (figure 6). This results in longer life for the animals.

The summary of 5 experiments, whereby immunization occurred orally on day 5 post inoculation shows that vaccinated animals have a mortality of 69.4% and controls 78.5%. The median time to death is the same for both groups, 11.2 days. The curves of figure 4 substantiate these statements. The difference in mortality between 69.4 and 78.5% is statistically not significant; therefore this does not represent a protection due to incubation time-vaccination. In the second model infection it also was apparent that a single, oral vaccination during the incubation period does not provoke, contrary to the subqutaneous route.

Provocation-effect in repeated, oral vaccination during incubation.

Because the three previous experiments did not alter the effects of a incubation-vaccination we next tried to show an effect by raising the dose of vaccine. In this way experiment 150 was done. Table 2

(rows 6 and 7) shows that 5 times the dose of vaccine(distributed from 3rd to 7th days post inoculation) raises mortality to unexpectedly high values. The pattern of mortality shows that in this case no provocation was present, as was observed for subqutaneously vaccinations. Mortality does not increase faster or earlier than in controls, but rather the large mortality difference occurrs slowly and days after the provocation vaccinations. The time-to-death is not shortened as usually, but rather extended (rows 9 and 10, table 2).

In figure 6 the first and largest experiment, 151, is represented about 5 parallel trials. Here animals were vaccinated during incubation once as well as 5x. The figure shows that the single vaccination has no influence; both mortality and mean time-to-death are the same. However, vaccinating 5x raised mortality 21%. Figure 6 again shows that no typical provocation existed, and mortality increases slowly and later than in both other curves.

Experiment 152 was different. This is presented in graph form in figure 7. The incubation and vaccination apparently protected the animals negatively; the singly vaccinated stronger than those vaccinated 5x. The repeated vaccinations may have damaged the animals somehow. Trial 153 shows very even values; mortality in the 5x was higher than in the 1x vaccinated group (table 2).

The mortality at the end of the trial is 83.5% for 5x vaccinated since whereas the controls were 17.5% lower. This is statistically significant. The curves of figure 8 show the slow rise, the cause of which is unknown. This seris of experiments assumes that the repeated incubation-vaccine is no indifferent, but somehow influences the course of the infection adversely.

Discussion

Our results cannot be properly compared to those of other authors, because the effectiveness of oral vaccination with inactivated virus has just been demonstrated (5). The results of earlier trials may be compared to other methods of vaccination and to other model systems. Our findings on two viral models support earlier work of S. typhinurium infection of mice, which showed that a single, effective, oral vaccination during the incubation time does not provoke enhancement (3). This further supports that oral vaccination is possible during epidemics and in times of catestrophy.

The difference between subqutaneously and oral vaccination becomes clearer, if we compare our previous data with subqutaniously vaccine after intercerebral infection with MEF, -polio (4) to those of oral vaccine in form of spidemic curves. This is presented in figure 9. On the left all experiments of report #5 (4) are presented. Vaccination parenterally and specific during incubation was compared to their controls. The curves (which represent 1136 mice studied) shows a clear provocation effect, which is emphasized by the shaded areas. Mortality was raised significantly from 69.1% to 77.9%; death time (average) lowered from 11.6 -10.3 days. The course of epidemic appears totally different in the right side of figure 9. In this case vaccination was oral, not parenterally. No provocation is apparent, but a small degree of protection.

They could be interpreted in that the repeated stimulation by antigenic substances other substances (interferon, complement, coproantibodies) are neutralized or used up. These latter compounds are needed by the mammalian organism to overcome infectious illness. This will have to be elucidated in further experimental work. It may be asked why the 5-fold dose of vaccine was not given on one day in order to effect a sudden change due to the excess of antigen. This would have required the feeding of an unphysiological dose of fluid (1.25 ml) during the incubation period. Specific changes are then difficult to define. Similarly for the Col. SK infection model this problem would have been difficult to standardize. The incubation period is so short that we would have been forced to give 5 vaccine doses in 1-2 days. This would have meant an injection of large doses of protein and lipids from the brain homogenate. It was previously reported that this does indeed alter the "resistance capability" of the experimental animals. Therefore defining specific and unspecific provocation would not have been possible.

Summary

1. If mice, orally infected with Col. SK virus, are orally immunized specifically with a heat-inactivated Col. SK vaccine, or nonspecifically with a formaldehyde-inactivated poliomyelitis vaccine, the pathological process is not influenced. In particular, the incubation immunization has no provocative action.

2. Oral incubation immunization with inactivated poliomyelitis vaccine, after intracerebral infection of mice with poliomyelitis virus, too, does not result in a provocation, as has been observed after parenteral incubation immunization.

3. Only a 5-fold increase of the immunization dose distributed over the incubation period causes an increase in mortality of the mice intracerebrally infected with poliomyelitis virus. However, for reasons as yet not clarified, this damage differs from that after parenteral incubation immunization. At the same time this effect of the overdose shows that oral immunization with inactivated virus is not indifferent.

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Versuch Nr.	Zeit des Versuches	Zahl der Mäuse	provoziert am ... Tag nach Infektion	Mortalität am Versuchsende in %: spezifische Provokation	Mortalität am Versuchsende in %: unspezifische Provokation	Mortalität am Versuchsende in %: Kontrolle	Mittlerer Sterbetermin in Tagen: spezifische Provokation	Mittlerer Sterbetermin in Tagen: unspezifische Provokation	Mittlerer Sterbetermin in Tagen: Kontrolle
* 1	2	3	4	5	6	7	8	9	10
144	Mai 65	100	2.	[illegible],0	—	56,0	2,62	—	3,11
[illegible]	Mai 65	[illegible]	[illegible]	[illegible]	—	[illegible]8,0	3,15	—	2,77
146	Juni 65	255	1.	60,0	55,2	51,7	2,55	2,45	2,33
133	Juni 65	99	1.	88,3	71,9	81,8	2,24	2,22	2,48
165	Juli 65	90	1.	76,7	60,0	76,7	2,25	2,60	2,35
Summe/Mittel	—	644	1.–2.	62,7		62,8	2,56		2,61
					61,7	62,4		2,40	2,40

Table 1

Oral vaccination during incubation with heat-inactivated Col. SK virus in brain suspension, or unspecific polio vaccine (salk) after oral Col. SK infection.

* 1-number, 2-time of experiment, 3-number of mice, 4-provocation day after infection, 5-specific provocation, 6-unspecific, 7-control, 8-specific, 9-unspecific, 10-control.

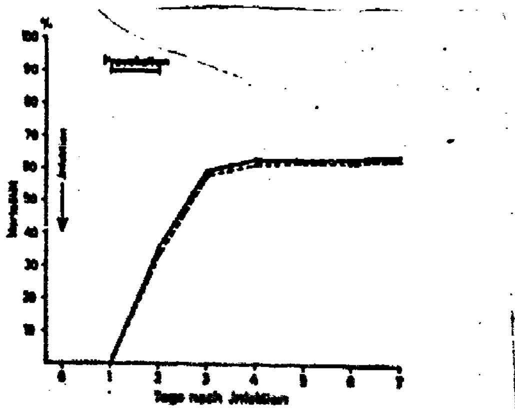

Figure 1.

Mortality vs.days of mice, orally infected with Col SK virus during incubation (see text for description). (specific oral heat-inactivated)

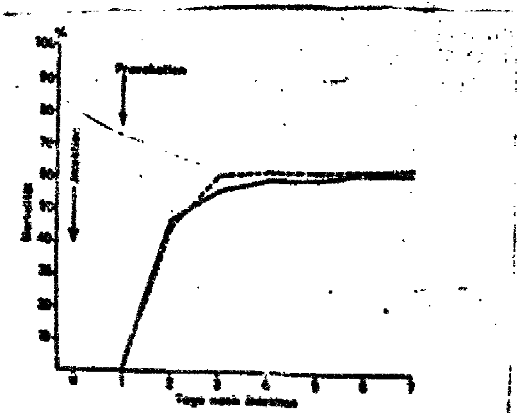

Figure 2.

Mortality vs. days of mice, orally infected with Col SK virus during incubation, unspecific oral with commercial, formaldehyde inactivated polio vaccine (solid = vaccinated; dashed = non vaccinated).

Versuch Nr.	Zeit des Versuches	Zahl der Mäuse	provoziert am ... Tag nach Infektion	Mortalität am Versuchsende in %: einmalige Provokation	Mortalität am Versuchsende in %: mehrfache Provokation	Mortalität am Versuchsende in %: Kontrolle	Mittlerer Sterbetermin in Tagen: einmalige Provokation	Mittlerer Sterbetermin in Tagen: mehrfache Provokation	Mittlerer Sterbetermin in Tagen: Kontrolle
* 1	2	3	4	5	6	7	8	9	10
141	April 66	77	5.	81.4	—	88,1	9,9	—	9,4
143	Mai 65	98	5.	79.2	—	82,0	10,9	—	10,8
150	Juli 65	100	3.–7.	—	84,0	34,0	—	11,8	10,8
~~151~~	~~Juli 66~~	~~300~~	5. od. 3.–7.	70,0	91,0	70,0	11,3	12,6	11,7
152	Aug. 66	100	5. od. 3.–7.	21,2	64,7	90,9	3,6	6,6	7,7
153	Sept. 66	137	5. od. 3.–7.	76,0	80,0	73,8	11,2	[illegible]	11,0
Summe/Mittel	—	912	—	69,4		78,5	11,2		11,2
					83,5	66,0		11,0	11,5

Table 2

Specific, oral incubation-vaccination with commercial polio vaccine, after IC infection with polio MEF.

* 1-number, 2-time of experiment, 3-number of mice, 4-provocation day after infection, 5-specific provocation, 6-unspecific, 7-control, 8-specific, 9-unspecific, 10-control.

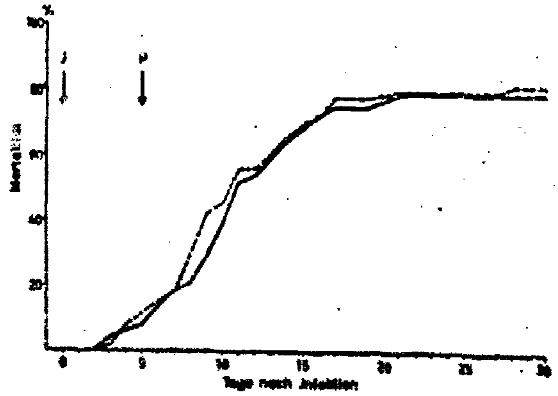

Figure 3

Mortality verses days in trial 143 as an example of no provocation of oral incubation-vaccine after IC infection with polio MEF. (solid-vaccinated; dashed - non vaccinated)

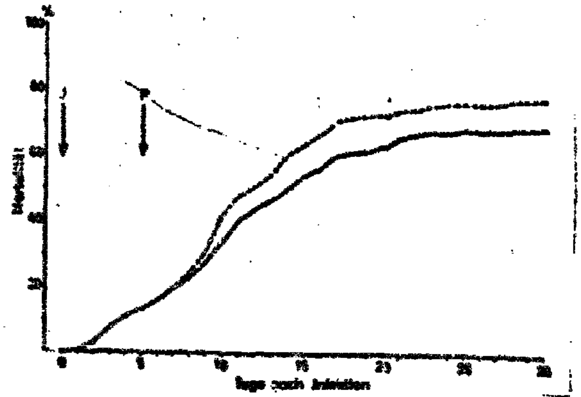

Figure 4

Mortality verses days of mice infected IC with polio MEF and immunized day 5 pi with formaldehyde inactivated polio vaccine (legend same as other figures.)

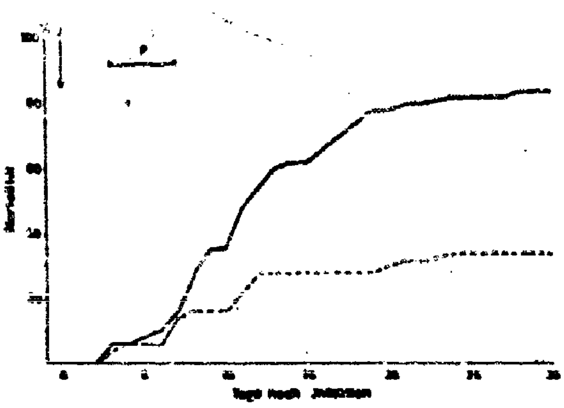

Figure 5

Mortality of trial 150; 5x oral specific incubation-vaccine after IC infection with polio.

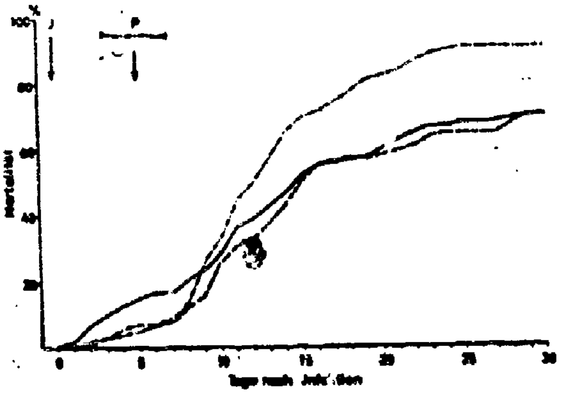

Figure 6

Mortality of trial 151; Difference between 1x and 5x oral specific vaccine after IC infection with polio virus (solid = 1x vaccinated; dot-dash = 5x; dashed= not vaccinated)

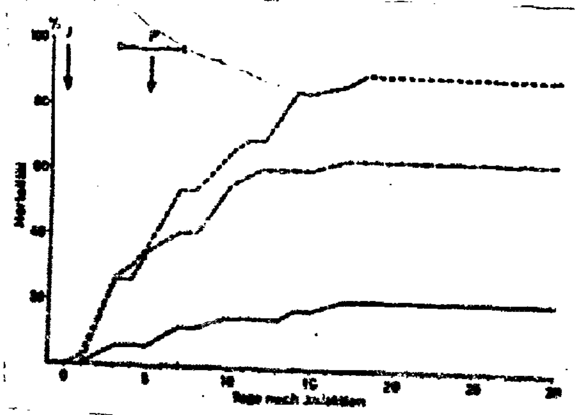

Figure 7

Mortality of trial 152; Example of 1x and 5x, oral specific vaccine after IC poliovirus. (legend as for figure 6)

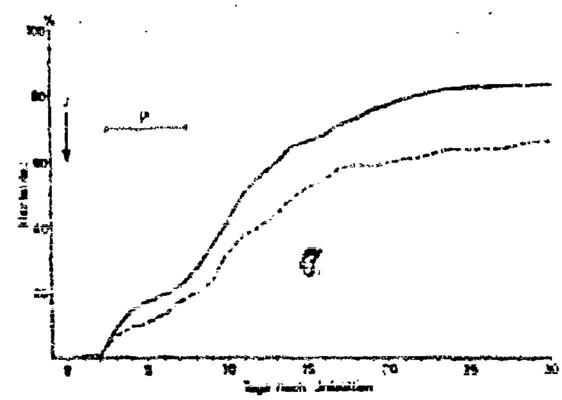

Figure 8

Mortality of mice infected IC with polio MEF and immunized days 3-7 pi 5x with formaldehyde in activated polio vaccine. (solid-vaccinated; dashed-non vaccinated)

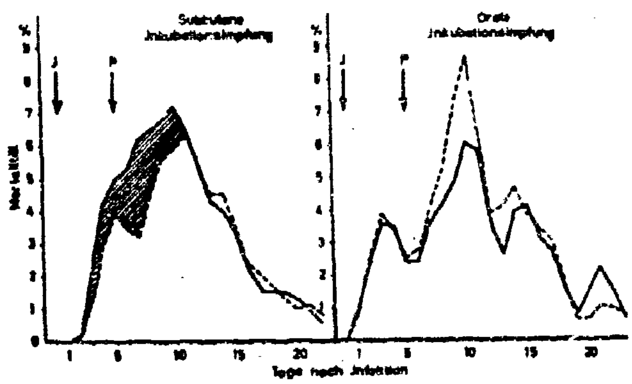

Figure 9

Comparing of subqutaneous and oral incubation-vaccine after IC infection of mice with poliovirus in the form of a epidemic curve. Left - Summary of all trials with parenteral vaccination from report 5 (4). Right - Conversion of curves from figure 4 for oral vaccination. (solid - vaccinated; dashed - non vaccinated; shaded area -provocation effect)